様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　４ 年　 ５ 月 ３１ 日現在

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研究課題名（和文）Enzymes as Active Matter at Nanoscales

研究課題名（英文）Enzymes as Active Matter at Nanoscales

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：本研究計画の遂行はCOVID-19の感染拡大に影響された。研究代表者はベルリンと金沢を拠点としており、自由に渡航できなくなった。しかし研究はほぼ予定通り進行し、酵素の活発な内部運動による流体力学的効果を解明した。まず、簡略化したダンベルモデルの解析により、触媒活性を持つ酵素の溶液における拡散の促進を正確に推定できた。また、酵素と周囲流体の運動量交換に基づく分子ダンス現象を提案し、その理論を構築した。これにより、酵素1分子に対する拡散増強効果を説明した。さらに、触媒活性を持つ酵素の濃厚溶液のガラス的性質を数値的に調べ、変形を伴う酵素活性がコロイドガラスの効果的な流動化をもたらすことを実証した。

研究成果の学術的意義や社会的意義
The project has led to a theoretical explanation for a family of experimental observations for diffusion enhancement in solutions of catalytically active enzymes. The obtained results are essential for the deeper understanding of reaction and diffusion processes in the living cells.

研究成果の概要（英文）：The execution of this project was affected by COVID-19 pandemic. The PI, based in Berlin and working part-time in Kanazawa, could not freely travel to Japan. Nonetheless, the research was to a large extent performed as planned. Hydrodynamic effects of active internal motions in mechano-chemical enzymes were explored. Analysis of simplified dimer models led to accurate estimates for diffusion enhancement in solutions of catalytically active enzymes. The phenomenon of molecular dancing, based on exchange of momenta between enzymes and surrounding fluid, was proposed, and its theory was developed. This has allowed us to explain the effects of boosted diffusion for single molecules of enzymes. Moreover, glass-like properties of dense solutions of catalytically active enzymes were numerically explored. It has been demonstrated that conformational activity of enzymes can have a strong effect on such colloidal glasses, leading to an effective fluidization of them.

研究分野：chemical and biological complex system

キーワード：enzymes　colloids　glasses　nonequilibrium　transport

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Bacteria or animals can autonomously change their shapes and, as a result, move themselves through the fluid (i.e., *swim*) and form active matter, or swarms. Molecular motors, such as myosin or kinesin, can also move themselves along filaments or microtubules; together with a polymer network they constitute active gels. But can biomolecules, like bacteria, behave as *free swimmers* and propel themselves through fluid by changing their shapes? Can dense colloids of active molecules, even *in absence of a polymer network*, already form active matter resembling a swarm? There has been important experimental evidence supporting this. S. Granick with coworkers [PNAS, 115, 14 (2018)] used superresolution optical microscopy to track motions of enzymes and found that, in presence of catalytic activity, their trajectories included episodes of rapid ballistic motion (leaps by tens of nanometers within about ten microseconds), as if these molecules could suddenly propel themselves.

C. Jacobs-Wagner with coworkers [Cell, 156, 183 (2014)] optically monitored motion of tracer nanoparticles in bacterial cytoplasm under normal physiological conditions and when metabolism (i.e., catalytic activity of cellular enzymes) was suppressed. Without metabolism, the cytoplasm exhibited glass-like properties characteristic for dense colloids. The metabolism however “fluidized” the cytoplasm, recovering classical transport properties and leading to diffusion enhancement by several orders of magnitude.

Self-propulsion of single enzyme molecules is a property that can be broadly used in biotechnology applications (see review [K. Dey, Angew. Chem. Int. Ed. 10.1002/anie.201804599 (2018)]), but its mechanisms were not understood. Transport properties in bacteria, such as diffusion and viscosity, are apparently dominated by non-equilibrium effects. Bacterial cytoplasm represents a crowded solution of enzymes, conformationally active under metabolism. While it was suggested by C. Jacobs-Wagner et al. that cytoplasm becomes fluidized through agitation by active biomolecules, the mechanisms of this behavior remained not clear.

２．研究の目的

The aim of this project was to theoretically investigate the effects of catalysis-induced conformational activity in enzyme proteins on individual motion of such molecules through the fluid and on transport phenomena in their crowded solutions (colloids).

３．研究の方法

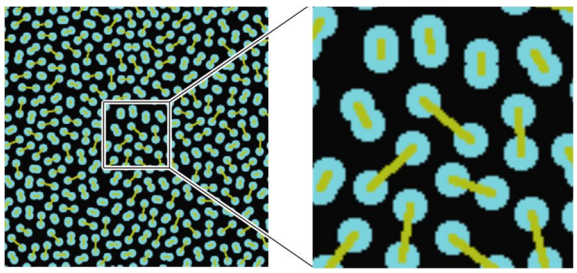
With the help of large-scale numerical simulations and extensive data analysis, we intended to develop a novel theory of active soft matter formed by enzymes. Thus, fundamental challenges in molecular biophysics of the cell had to be addressed and perspectives for control and steering of transport processes in actual living cells and in synthetic biotechnological systems would have been opened.

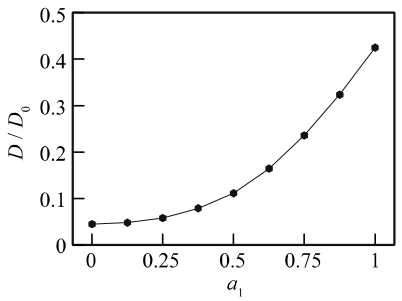
４．研究成果

The execution of this research program has been affected by the COVID-19 pandemic. The PI, based in Berlin and employed part-time in the Kanazawa university, could not regularly travel to work in Japan and meet with the co-PIs. Therefore, some of the project goals have not been reached. Nonetheless, a large part of the program has been implemented by following the original plan. Below we summarize the main results.

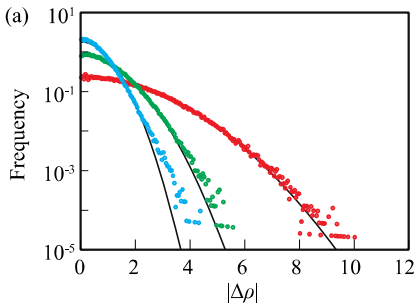
A. Crowded enzyme solutions as active glass-like colloids.

Crowded solutions of mechanochemical catalytically active enzymes were modeled as dense active colloids (Fig. 1) of oscillating dimers (dumbbells). The beads in a dumbbell are connected by an elastic link whose natural length changes periodically with time. There are repulsive interactions between the dumbbells. The Langevin description, including thermal noise but neglecting hydrodynamic effects, is employed. Numerical simulations for such systems have been performed. The model parameters were chosen in such a way that, in absence of active oscillations, the passive colloid behaved as a glass: diffusion of particles was suppressed, structural relaxation time was strongly increased and deviations from the Gaussian distribution for particle displacements were observed.





When active oscillations were introduced and their amplitude a_1 was gradually increased, *fluidization* of the glass was taking place. The diffusion became enhanced (Fig. 2), the relaxation time became shorter and the Gaussian displacement distribution, characteristic for classical Fickian diffusion, became recovered (Fig.3). Remarkably, such transition has been found in the parameter region typical for bacterial cytoplasm. Thus, the hypothesis based on the experimental data has been theoretically confirmed. Our reported simulations [EPL 128, 40003 (2019)] were for relatively small systems that were however already sufficient to demonstrate the principal effects. Simulations for large systems have been undertaken at the final stage of the project; their results and the detailed analysis are being prepared for publication.



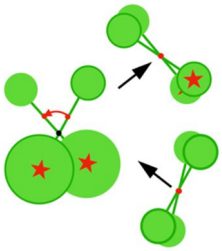
B. Hydrodynamic effects of mechanochemical enzymes.

Conformational changes, that accompany catalytic turnover cycles, induce circulating hydrodynamic flows in the solution around enzymes. Previously, it has been shown that non-equilibrium fluctuating flows, collectively generated by enzymes, lead to additional mixing and can enhance diffusion of passive particles (tracers) in the solution. However, reliable estimates for such phenomena were not possible in absence of the detailed analysis of hydrodynamic effects of individual enzymes. In the framework of the project, such analysis has been performed [Soft Matter 16, 10734 (2020)]. At a sufficient separation, enzymes can be viewed as oscillating hydrodynamical dipoles. We have analytically and numerically investigated the intensity and statistical properties of such dipoles within the minimal model active dimer model of a mechanochemical enzyme. This has allowed us to obtain much more precise estimates for diffusion enhancement of passive particles in weak solutions of catalytically active enzymes.

C. Molecular dancing and boosted diffusion of enzymes.

In the experiments, boosted diffusion of single active enzymes has been repeatedly observed. This behavior, involving individual molecules, cannot be described by the above collective effects. Following previous

literature, we have initially expected that boosted diffusion should be a consequence of hydrodynamic self-propulsion effects. However, the detailed analysis performed in this project has revealed thar such explanation does not hold because the molecular propulsion velocities are too small. Hence, a different explanation had to be sought. As we have found, all principal experimentally observed aspects of boosted single-enzyme diffusion can be reproduced within the novel theory of molecular dancing proposed by us.



Because of the momentum exchange with the surrounding fluid, the center of mass of an enzymes performs motions within a turnover cycle. These motions are reciprocal and therefore they do not lead to net self-propulsion. However, if rotational diffusion is so fast that the enzyme tumbles within each cycle, the forward and reverse motions do not compensate one another (Fig. 3). As a result, an additional non-equilibrium random walk is performed, leading to diffusion enhancement of the enzyme. The trajectory of a molecule represents a combination of rapid leaps and more slow classical diffusive motion between them (Fig. 4). Moreover, in agreement with the experimental data, the boosted diffusion is linearly dependent on the rate of energy supply. A detailed analytical theory of such phenomena has been constructed and numerical simulations have been carried out. This last study has been undertaken at the final stage of the project and its results are being prepared for publication.

Thus, we have **found answers** to the two main questions formulated at project's start. *(i)No, enzymes practically cannot swim, contrary to conjectures previously made. However, the diffusion of active enzymes can indeed become boosted, as experimentally observed, due to the effects of dancing based on mechanical momentum exchange. (ii)Yes, conformational activity in enzymes in crowded solutions, such as cytoplasm, leads to fluidization of such glass-like systems.*

５．主な発表論文等

〔雑誌論文〕　計8件（うち査読付論文　8件／うち国際共著　4件／うちオープンアクセス　2件）

１．著者名 Koyano Yuki、Kitahata Hiroyuki、Hasegawa Koji、Matsumoto Satoshi、Nishinari Katsuhiro、Watanabe Tadashi、Kaneko Akiko、Abe Yutaka	４．巻 102
２．論文標題 Diffusion enhancement in a levitated droplet via oscillatory deformation	５．発行年 2020年
３．雑誌名 Physical Review E	６．最初と最後の頁 033109/1～10
掲載論文のDOI（デジタルオブジェクト識別子） 10.1103/PhysRevE.102.033109	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Kitahata Hiroyuki、Koyano Yuki	４．巻 89
２．論文標題 Spontaneous Motion of a Camphor Particle with a Triangular Modification from a Circle	５．発行年 2020年
３．雑誌名 Journal of the Physical Society of Japan	６．最初と最後の頁 094001/1～10
掲載論文のDOI（デジタルオブジェクト識別子） 10.7566/JPSJ.89.094001	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Era Katsutomo、Koyano Yuki、Hosaka Yuto、Yasuda Kento、Kitahata Hiroyuki、Komura Shigeyuki	４．巻 133
２．論文標題 Autonomous elastic microswimmer	５．発行年 2021年
３．雑誌名 EPL (Europhysics Letters)	６．最初と最後の頁 34001/1～7
掲載論文のDOI（デジタルオブジェクト識別子） 10.1209/0295-5075/133/34001	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Al-Izzi Sami C.、Sens Pierre、Turner Matthew S.、Komura Shigeyuki	４．巻 16
２．論文標題 Dynamics of passive and active membrane tubes	５．発行年 2020年
３．雑誌名 Soft Matter	６．最初と最後の頁 9319～9330
掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/D0SM01290D	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Hosaka Yuto、Komura Shigeyuki、Mikhailov Alexander S.	4．巻 16
2．論文標題 Mechanochemical enzymes and protein machines as hydrodynamic force dipoles: the active dimer model	5．発行年 2020年
3．雑誌名 Soft Matter	6．最初と最後の頁 10734～10749
掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/D0SM01138J	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Flechsig Holger、Mikhailov Alexander S.	4．巻 16
2．論文標題 Simple mechanics of protein machines	5．発行年 2019年
3．雑誌名 Journal of The Royal Society Interface	6．最初と最後の頁 20190244
掲載論文のDOI（デジタルオブジェクト識別子） 10.1098/rsif.2019.0244	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

1．著者名 Koyano Y.、Kitahata H.、Mikhailov A. S.	4．巻 128
2．論文標題 Diffusion in crowded colloids of particles cyclically changing their shapes	5．発行年 2020年
3．雑誌名 EPL (Europhysics Letters)	6．最初と最後の頁 40003
掲載論文のDOI（デジタルオブジェクト識別子） 10.1209/0295-5075/128/40003	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Hosaka Yuto、Komura Shigeyuki、Andelman David	4．巻 101
2．論文標題 Shear viscosity of two-state enzyme solutions	5．発行年 2020年
3．雑誌名 Physical Review E	6．最初と最後の頁 12610
掲載論文のDOI（デジタルオブジェクト識別子） 10.1103/PhysRevE.101.012610	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計6件（うち招待講演　3件／うち国際学会　1件）

１．発表者名
北畑裕之

２．発表標題
液滴中の振動流による拡散増強のモデル

３．学会等名
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４．発表年
2020年

１．発表者名
江良勝智，小谷野由紀，保阪悠人，安田健人，北畑裕之，好村滋行

２．発表標題
自律的に速度を選択する弾性スイマー

３．学会等名
日本物理学会 2020年秋季大会

４．発表年
2020年

１．発表者名
Alexander S. Mikhailov

２．発表標題
SIMPLE MECHANICS OF PROTEIN MACHINES

３．学会等名
20th RIES-HOKUDAI International Symposium（招待講演）

４．発表年
2020年

１．発表者名
江良勝智，小谷野由紀，保阪悠人，安田健人，北畑裕之，好村滋行

２．発表標題
自律的に速度を選択する弾性スイマーII

３．学会等名
日本物理学会 第76回年次大会

４．発表年
2021年

１．発表者名
Alexander S. Mikhailov

２．発表標題
Simple Mechanics of Protein Machines

３．学会等名
The 20th RIES-HOKUDAI International Symposium（招待講演）（国際学会）

４．発表年
2019年

１．発表者名
小谷野 由紀, Alexander S. Mikhailov, 北畑 裕之

２．発表標題
周期的な形状変化を行うアクティブダンベル状粒子集団内における拡散促進

３．学会等名
日本物理学会第75回年次大会

４．発表年
2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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研究分担者	小谷野　由紀 (Koyano Yuki) (50849643)	神戸大学・人間発達環境学研究科・助教 (14501)	

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計1件

国際研究集会	開催年
Joint UBI-NanoLSI workshop “TRENDS IN MOLECULAR BIOPHYSICS OF LIVING CELLS”	2019年～2019年

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関